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# Diagnostic Value of Lactoferrin Analysis in Pleural Effusions

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**Summary:** Lactoferrin was analysed with an ELISA in pleural effusions from 21 patients with malignant exudative effusions (15 carcinomas and 6 mesotheliomas), 12 patients with non-malignant exudative effusions of unknown aetiology, 11 patients with transudative effusions due to congestive heart failure, 12 patients with exudative effusions secondary to infection, and 2 patients with tuberculous effusions. Median pleural fluid lactoferrin was 133 μg/l (range 25–435) in carcinomas, 55 μg/l (23–185) in mesotheliomas, 198 μg/l (31–530) in non-malignant exudates, 68 μg/l (17–205) in transudates, 1815 μg/l (1380–2050) in infectious exudates and 107 μg/l (88–125) in tuberculosis. Due to a wide overlap between the various groups pleural fluid lactoferrin appears to be of limited value in the routine diagnostic evaluation of non-infectious pleural effusions, but seems to separate infectious exudates from non-infectious exudates.

## Introduction

Lactoferrin is an iron-binding glycoprotein which is very similar in structure to transferrin (1). Lactoferrin is present in the specific granules of neutrophilic granulocytes (2), plasma (3), milk (4) and various other secretions (5). Elevated concentrations of lactoferrin has been found to be indicative of inflammation in several clinical conditions (6–8).

The most frequently examined pathological effusion is probably pleural fluid, in which lactoferrin has not previously been investigated. Considering that lactoferrin has been found in bronchial mucus (9) and lung cancer extracts (10) it is possible that the level of lactoferrin in pleural fluid may be of diagnostic value. Especially high values could be expected in effusions secondary to bacterial infections, but not in effusions secondary to viral infections, since the neutrophil content of lactoferrin has been shown to be decreased in viral infections (11). The purpose of the present study was to demonstrate lactoferrin in pleural fluid and to assess the value of lactoferrin in the differential diagnosis of pleural fluids of various origins.

## Patients and Methods

The study comprised 58 patients (21 females, 37 males) with a median age of 68 years (range 27–87), in whom a diagnostic thoracocentesis was performed. The final diagnoses were decided after observing the clinical course of the patients in a follow-up period after entry into the study (12–24 months), and after diagnostic procedures including cytological examination and culture of pleural fluid for bacteria including mycobacteria (but not for viruses), and histological examination of pleural biopsy specimens obtained by thoracoscopy or at autopsy. The patients were divided into six diagnostic groups.

*Group 1, carcinomatous exudates*

Fifteen patients had malignant disease with pleural invasion (11 pulmonary carcinomas and 4 extrapulmonary carcinomas with pleural metastases).

*Group 2, mesothelioma exudates*

Six patients had malignant mesothelioma.

*Group 3, idiopathic exudates*

Twelve patients without congestive heart failure or any other obvious cause of pleurisy were classified as having pleural effusions of unknown aetiology.

*Group 4, heart failure transudates*

Eleven patients with congestive heart failure and pleural effusions responding to diuretic treatment, who did not have any other obvious cause of pleurisy, were classified as having transudates.

*Group 5, infectious exudates*

Twelve patients with pleural exudates secondary to pulmonary infection. Six patients had fever, a pulmonary infiltrate and clear or turbid yellow pleural fluid, all of which disappeared after antibiotic treatment, and 6 patients had empyema which disappeared after antibiotic treatment and intermittent thoracocentesis. Pathogenic bacteria were found in 8 cases (*Streptococci* 4, *Staphylococcus aureus* 2, *Haemophilus* 2). In 4 cases treated with antibiotics at the time of cultivation no bacteria were found.

*Group 6, tuberculous exudates*

Two patients had a positive culture of *M. tuberculosis* in the pleural fluid.

### Sampling

Pleural fluid was collected in heparin coated glass tubes and centrifuged within one hour. The pleural fluid was then at once deep frozen and kept at −18 °C until use.

The number of neutrophils was estimated arbitrarily in three groups (none (0), moderate (+, ++), many (+++) by a pathologist.

The following analyses were performed on centrifuged pleural fluid: Protein by the biuret reaction (12) and lactoferrin by an ELISA method. The ELISA method has been described in detail previously (13).

### Assay of lactoferrin

One-hundred µl of prediluted lactoferrin antiserum (DAKO Immunoglobulins Ltd., Copenhagen, Denmark) was pipetted into each well of a microtitration plate and left overnight at room temperature. The following day the wells were emptied by inversion and tapped dry on absorbent paper. The plate was then washed three times with wash buffer. Lactoferrin standard (Behringwerke AG-Marburg, Germany) or 100 µl of test samples diluted 1 : 100 with dilution solution were applied as duplicates in the wells of the microtitration plate and incubation at room temperature. The wells were emptied after 60 min and washed three times with wash buffer. One-hundred µl of prediluted anti-human lactoferrin (DAKO Immunoglobulins Ltd., Copenhagen, Denmark) was then pipetted into each well and left for 60 min at room temperature. The wash procedure was repeated and 100 µl of a colour reagent applied to each well. The reaction was now allowed to proceed for 15 min, before being stopped by the addition of 200 µl of sulphuric acid. The extinction of the contents of each well was measured in a Titertec Multiscan MC (Flow Laboratories) at 488 nm. A dose-response curve was plotted on semilogarithmic paper. At lactoferrin concentrations between 25 µg/l and 345 µg/l the intra-assay coefficient of variation varied between 2% and 4% and the inter-assay coefficient of variation varied between 3% and 14%, being highest at the lower concentrations.

### Statistical analysis

Due to sample size the results of group 6 were not analysed statistically. Differences between groups were evaluated by the *Kruskal-Wallis* test and *Wilcoxons* test for unpaired differences.

## Results

Figure 1 shows the concentrations of lactoferrin in the subgroups. Pleural fluid lactoferrin is much higher in the infectious group than in the other groups ($p < 0.0001$). Among the 12 infectious patients there was no difference between those with clear or turbid yellow fluid and those with empyema. The other groups did not differ significantly from each other. Figure 2 shows the concentrations of protein in the subgroups. As expected the level of protein in transudates differ significantly from the level of protein in exudates ($p < 0.0001$). The exudate groups, however, do not differ from each other. The

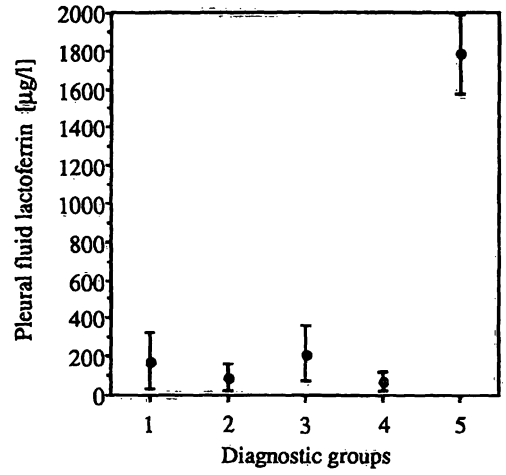


**Fig. 1** The concentration of pleural fluid lactoferrin (mean, 1 standard deviation) in the subgroups.
Group 1: carcinomatous exudates:
Group 2: mesothelioma exudates;
Group 3: idiopathic exudates;
Group 4: heart failure transudates;
Group 5: infectious exudates.

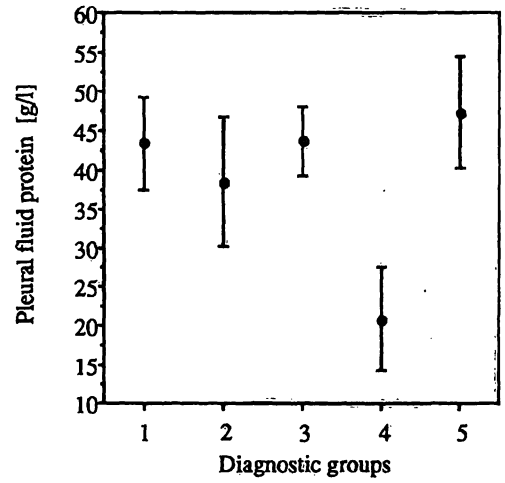


**Fig. 2** The concentration of pleural fluid protein (mean, 1 standard deviation) in the subgroups.
Group 1: carcinomatous exudates;
Group 2: mesothelioma exudates;
Group 3: idiopathic exudates;
Group 4: heart failure transduates;
Group 5: infectious exudates.

**Tab. 1** Pleural fluid analyses in patients with effusions of malignant, benign and infectious origin, and in transudates and non-infectious exudates; median (range).

| Pleural effusion | Group | Lactoferrin (μg/l) | Protein (g/l) |
|---|---|---|---|
| Malignant (N = 21) | 1 + 2 | 89 (23–435) | 42 (24–53) |
| p | | NS | 0.03 |
| Benign (N = 23) | 3 + 4 | 95 (17–530) | 32 (11–50) |
| p | | 0.0001 | 0.0001 |
| Infectious (N = 12) | 5 | 1815 (1380–2050) | 47 (38–59) |
| p | | 0.0001 | NS |
| Exudate (N = 33) | 1 + 2 + 3 | 150 (23–530) | 44 (24–53) |
| p | | 0.04 | 0.0001 |
| Transudate (N = 11) | 4 | 68 (17–205) | 22 (11–30) |

levels of lactoferrin in malignant and benign effusions were almost identical (tab. 1). Pleural fluid lactoferrin is significantly higher in exudates than in transudates (tab. 1). The pattern of differences between groups is similar to that of pleural fluid protein, although less significant (tab. 1). Figure 3 shows that the level of lactoferrin is higher in the group with many neutrophils than in the groups with none or a moderate number of neutrophils ($p < 0.0001$). There were many neutrophils in all 12 exudates from the infectious group. In the other patient groups there were either none or a moderate number of neutrophils. The concentration of pleural fluid protein was not different in the three groups.

Pleural fluid lactoferrin in the two patients with tuberculosis was 88 μg/l and 125 μg/l, respectively.

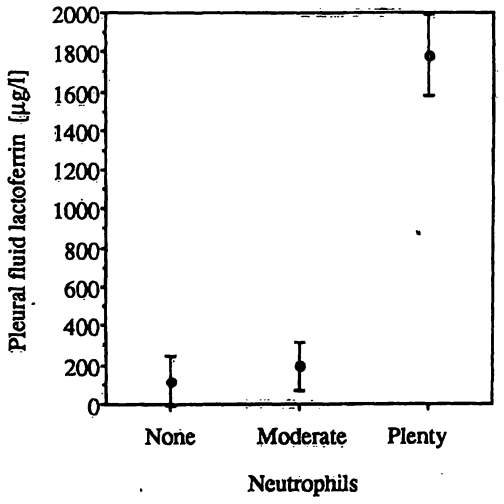


**Fig. 3** The concentration of pleural fluid lactoferrin (mean, 1 standard deviation) according to number of pleural fluid neutrophils.

## Discussion

The present results indicate that lactoferrin completely separates infectious effusions of probable bacterial origin (with the exception of tuberculosis) from non-bacterial effusions. Lactoferrin cannot be used to distinguish between malignant and non-malignant effusions (tab. 1). The significant differences between the group of patients with transudates and the group with exudates seem clinically unimportant, due to considerable overlap between the groups (tab. 1), except that values > 200 μg/l were found almost exclusively in idiopathic and carcinomatous exudates, and not in heart failure transudates or mesothelioma exudates. This is probably explained by the fact that neutrophilic granulocytes were present in more than 50% of idiopathic and carcinomatous exudates but in less than 20% of transudates and mesothelioma exudates, in accordance with the positive relation found between pleural fluid neutrophils and pleural fluid lactoferrin (fig. 3). The high lactoferrin level in bacterial effusions is also due to a high number of neutrophils. A positive correlation between the number of neutrophils and the level of lactoferrin has been found in cerebrospinal fluid (6) and synovial fluid (7), reflecting the fact that lactoferrin is produced in the granules of the neutrophils. In another study *Rosenmund* et al. found that the plasma lactoferrin/neutrophil ratio was relatively constant in conditions with a high lactoferrin level such as cirrhosis of the liver and septicaemia, as well as in conditions with a low level of lactoferrin such as tumours, iron deficiency and hepatitis B, strongly indicating that the lactoferrin level depends mainly on the neutrophil count (14).

There were many neutrophils in all 12 exudates from the infectious group, but only 6 of these were purulent (empyema). Pus contains not only intact neutrophils, but also liquefied necrotic material and disintegrated neutrophils, which contributes to the white creamy appearance of empyema. The non-purulent parapneumonic effusions may be clear or turbid, depending on the number of neutrophils. Such effusions cannot macroscopically be distinguished from pleural effusions of non-infectious origin. Normally the non-purulent effusions are uncomplicated and disappear after antibiotic treatment, whereas empyemas are complicated and need drainage (15). Since we found no difference in lactoferrin in these two subgroups, this may indicate that the number of neutrophils in the two groups was the same, or that the neutrophil content of lactoferrin was different. Since we did not measure the specific number of neutrophils, we are not able to elucidate this problem further. The median level of lactoferrin in non-bacterial pleural fluid (92 μg/l) (range 17–530) is relatively low compared with that found in synovial fluid in patients with os-

teoarthrosis (970 μg/l ± 390, mean ± SEM) (7) and normal plasma (median 395 μg/l, range 190–810) (8), but higher than in normal cerebrospinal fluid (7.3 μg/l ± 4.7, mean ± SEM) (6). Probably lactoferrin in pleural fluid originates entirely from neutrophils and/or plasma, and not from the pleura itself. The level of lactoferrin in pleural exudate due to bacterial infection in the present study was higher than found in cerebrospinal fluid from patients with meningitis (360 μg/l ± 168, mean ± SEM) (6), but not as high as found in synovial fluid from patients with septic arthritis (127 000 μg/l ± 46 100, mean ± SEM) (7). The relatively low level of lactoferrin found in tuberculous pleural fluids corresponds well with the fact that the tuberculous inflammation consists mainly of mononuclear cells and lymphocytes and not of neutrophils.

In conclusion, this study has shown that lactoferrin is present in pleural effusions. The value of lactoferrin in the differentiation between pleural effusions of non infectious and of infectious origin seems high. Although values higher than 200 μg/l are rare in transudates and exudates from mesotheliomas, lactoferrin is of limited value in the differentiation between non-infectious pleural effusions of different origin. Protein is still the best discriminator between transudates and exudates, but none of the investigated variables could be used to differentiate between exudates of non-infectious origin. In our investigation we measured lactoferrin in empyemas for comparison. In clinical practice it shall not be necessary to measure lactoferrin in purulent effusions, since these almost always are complicated parapneumonic empyemas. The main result of our investigations is that a clear or turbid yellow fluid with a high level of lactoferrin is probably of bacterial origin, whereas one with a low level of lactoferrin is probably non-bacterial.

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